

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

THIS PAGE BLANK (USPTO)



The
Patent
Office

PCT/GB 99/04027

01 DECEMBER 1999

INVESTOR IN PEOPLE

091857091

GB99/4027

4

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH
RULE 17.1(a) OR (b)

The Patent Office

Concept House

Cardiff Road

Newport

South Wales

NP10 8QQ

RECD 26 JAN 2000

WIPO

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the "public limited company" part of the name or the last part of the name of the words "public limited company", references to the name of the company shall be treated as references to the name

*Mark A
Rolder*

In accordance with the

"public limited company" may be replaced by p.l.c.,

re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed *Andrew Garside*
Dated 11 January 2000

THIS PAGE BLANK (USPTO)

B

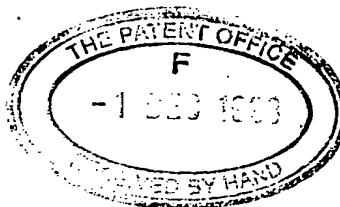
1/77

02DEC98 E408892-1 D02732
F01/7700 0.00 - 9826378.3

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH**Request for grant of a patent**

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)



1. Your reference

A1109

2. Patent application number

(The Patent Office will fill in this part)

9826378.3

- 1 DEC 1998

3. Full name, address and postcode of the or of each applicant (*underline all surnames*)

(1) ABERDEEN UNIVERSITY; AURIS BUSINESS CENTRE, 2 ST MACHAR DRIVE, ABERDEEN AB2 1RY, UNITED KINGDOM; AND (2) SCOTTISH NATIONAL BLOOD TRANSFUSION SERVICE, TRINITY PARK HOUSE, SOUTH TRINITY ROAD, EDINBURGH EH5 3SE

Patents ADP number (*if you know it*)

① 293951002

If the applicant is a corporate body, give the country/state of its incorporation

② 7559883001

4. Title of the invention

ALLO-REACTIVE T-CELL EPITOPE

5. Name of your agent (*if you have one*)

ABLETT & STEBBING
CAPARO HOUSE
101-103 BAKER STREET
LONDON
W1M 1FD

"Address for service" in the United Kingdom to which all correspondence should be sent (*including the postcode*)

6551001

Patents ADP number (*if you know it*)6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (*if you know it*) the or each application number

Country

Priority application number
(*if you know it*)Date of filing
(*day / month / year*)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(*day / month / year*)8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (*Answer 'Yes' if:*

YES

- a) *any applicant named in part 3 is not an inventor, or*
- b) *there is an inventor who is not named as an applicant, or*
- c) *any named applicant is a corporate body.*

See note (d)

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form.
Do not count copies of the same document

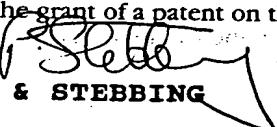
Continuation sheets of this form	0
Description	13
Claim(s)	2
Abstract	0
Drawing(s)	2

10. If you are also filing any of the following, state how many against each item.

Priority documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent (Patents Form 7/77)	0
Request for preliminary examination and search (Patents Form 9/77)	0
Request for substantive examination (Patents Form 10/77)	0
Any other documents (please specify)	0

11.

I/We request the grant of a patent on the basis of this application.

Signature 
ABLETT & STEBBING

Date **1 December 1998**

12. Name and daytime telephone number of person to contact in the United Kingdom

GK ABLETT/PJH STEBBING (0171-935-7720)

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.
- Write your answers in capital letters using black ink or you may type them.
- If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- Once you have filled in the form you must remember to sign and date it.
- For details of the fee and ways to pay please contact the Patent Office.

ALLO-REACTIVE T-CELL EPITOPES

The present invention relates the mapping of allo-reactive T-cell epitopes on the rhesus(RhD and RhCE) protein and to the 5 use of such epitopes to modulate immune response, particularly in RhD negative women.

From WO 91/07492, it is known to provide DNA sequences encoding complementary determining regions of variable domains 10 of human anti-RhD antibodies and their use in the production of antibody molecules.

Similarly WO 97/49809 reveals polypeptides capable of forming antigen binding structures specific for rhesus D antigens. 15 Neither of these two documents reveal the immune response of the fragments of the present invention.

The full amino acid sequence of the RhCE polypeptide and the differences in sequence for c, e and D polypeptides is shown 20 in Figure 1 hereinafter.

It is the case that during pregnancy, and especially during parturition, women who are RhD negative but who carry RhD positive fetuses risk being immunized by the RhD protein blood 25 cells of their own baby.

This is because women as mothers produce anti-D antibodies which cross the placenta and cause Rh haemolytic disease in any subsequent RhD positive pregnancies. Such haemolytic 30 disease can be fatal for the neonate.

Currently purified anti-D immunoglobulin is injected whenever a mother is exposed to fetal RhD positive red blood cells

which may occur during e.g., amniocentesis, antepartum haemorrhage but mainly at parturition. About 17% of Caucasian women are RhD negative so that most industrialized countries have RhD prevention programmes wherein all RhD negative women 5 receive prophylactic anti-D at delivery or in association with the other high risk events alluded to above. Further in many countries, routine antepartum prophylaxis to minimize the instance of Rh haemolytic disease is practised.

10 There are a number of problems with this approach. In the first place efficacy is never entirely complete since events can be missed or undeclared or a foetal haemorrhage can be larger than the anti-D can neutralize. Secondly current anti-D immunoglobulin comes from deliberately immunized donors 15 which puts volunteers, often male (paid or not) at some small risk. Partly for this reason there is a worldwide shortage of anti-D immunoglobulin. Finally there are concerns about the safety of recipients who may be exposed to transfusion transmitted infections such as by inadvertent infection with 20 agents such as CJD for which there is no satisfactory test.

It is known that mammals may be tolerized to certain stimuli by taking in specific peptide fragments, for example from the nasal mucosa or via the gut. We have now found that a good 25 way of abolishing the immune response to RhD in recipient females prior to, during, or after pregnancy is to administer RhD or RhCE peptides via the mucosa such as the nasal, buccal, or anal mucosa or transdermally. The peptide fragments in accordance with the present invention will then enter mucosal 30 cells and effectively immunize the subject without causing a full blown antigenic reaction which may well be the case should the peptide fragments of the present invention reach circulating blood system at the first instance.

The outcome of this approach is to develop a "vaccine" using Rh peptides which will suppress the immune response to RhD and RhCE proteins.

5 Major advances have been made in the last three decades in the understanding of cellular interreactions in immune responses. These are included in the discovery that helper T-lymphocytes recognize short peptide fragments that have been processed from protein antigens and displayed bound to MHC Class II molecules by specialized antigen presenting cells. With the growing realization of the role played by helper T-lymphocytes in driving both protective and damaging immune responses, attention has been focussed on approaches to specifically stimulate or regulate these cells.

15

One approach which has been successful in controlling auto-aggressive T-lymphocytes in animal models of autoimmune disease is to map the antigen-derived fragments that they recognize (epitopes) and to administer synthetic peptides of 20 identical or related sequence *in vivo*. In general, the peptides of wild-type sequences are effective tolerogens only if given by the mucosal or transdermal routes, whereas analogue sequences with particular amino-acid substitutions can also modulate the immune response when injected.

25

The opposite approach which is relevant in the field of vaccine design is to inoculate the peptides that T-lymphocytes recognize from foreign microbial proteins in a form (usually plus adjuvant) that can stimulate a protective 30 immune response. We have used the above two approaches to modulate the immune response to an important blood group antigen RhD.

The RhD antigen is a transmembrane protein consisting of 417 amino acids with 12 putative transmembrane domains and 6 extra cellular loops. A series of peptides have been constructed in the present invention based on the RhD protein each being 5 15 AA long, and tested *in vitro* against T-lymphocytes from normal individuals and donors who have been alloimmunized to produce anti-D.

Certain RhD peptides have been found to specifically stimulate 10 the helper-T cells of alloimmunized individuals. There is therefore a correlation between HLA-DR type alloimmunized donors and the peptides which stimulate helper-T cell responses.

15 In summary therefore, we have mapped helper-T cell epitopes on the RhD protein. The characterization of a helper epitope that is targeted in most alloimmunized donors and the identification of correlations between HLA-DR type and particular dominant epitopes opens the way for the evaluation 20 of peptide immunotherapy as a novel way to regulate the immune response to RhD and to prevent Rh haemolytic disease and anti-D related transfusion problems. According therefore to one aspect of the present invention, there is provided a composition adapted for the prevention of alloimmunization of 25 a subject said composition comprising an immunologically effective epitope of RhD or RhCE proteins or an immunologically active analogue thereof.

Currently, anti-D which is given to pregnant women during 30 significant events in pregnancy may be considered as a passive form of immunotherapy because it has the effect of blocking the effects of immune events on a temporary basis.

The replacement of passive with active peptide immunotherapy in RhD negative women is an attractive option since safe synthetic tolerogens can be developed and given before pregnancy thus avoiding foetal exposure. Suppression throughout pregnancy would mean that only one injection was necessary, considerably simplifying management of RhD negative women and also it may be possible for the first time to reverse rather than prevent alloimmunization by administration of tolerogenic peptides to individuals who already have produced anti-D with the objective of "switching-off" the immune response to RhD.

Accordingly the categories of individual to whom prior immunization would be considered are as follows:-

- 15 (1) All RhD negative women during their child bearing years; and
- (2) RhD negative regular recipients of blood products; who might be exposed to RhD positive blood products for example haemological malignant disease, sickle cell

20 disease and thalassaemia.

The use of synthetic peptides in accordance with the present invention thus removes concerns about viral infection being transmitted by blood cells from volunteer recipients, but it is the time consuming and expensive procedures required to validate accredited donors and donations that are important too.

According to a further aspect of the present invention, there is provided a composition adapted for the induction of alloimmunization of a volunteer, said composition comprising an immunologically effective epitope of an RhD or RhCE protein

or an immunologically active analogue thereof disposed in a pharmacologically acceptable injectable vehicle.

By use of these compositions, volunteers who are usually men,
5 can avoid the usual injection of red blood cells.

According to another aspect of the present invention, there
is provided a tolerizing peptide fragment disposed in a
pharmacologically effective vehicle, said vehicle being
10 adapted for non-injection administration to the subject. Such
a vehicle may be adapted for transdermal or transmucosal
administration or the vehicle may be formulated with an
enteric coating for oral administration. Such tolerizing
peptide fragments may specifically include those RhD fragments
15 given above.

The present invention will now be described by way of
illustration only with reference to the accompanying
drawings:-

20

Figure 1 shows the full amino acid sequence of RhCE polypeptides. Differences in the sequence for c, e and D polypeptides are also shown.

25 Figure 2 shows the distribution of stimulatory RhD peptides in anti-RhD immunoglobulin donors.

EXAMPLE

30 Two complete panels of 42 15-mer peptides, with 5 amino acid overlaps, were synthesized (Multiple Peptide Service, Cambridge Research Biochemicals, Cheshire, UK), corresponding to the sequences of the 30kD Rh proteins associated with

expression of the D or Ce/Ec blood group antigens respectively (ie D peptides 1-42 and Cc/Ee peptides 1-42). The amino acid sequences for each of these proteins were deduced independently from cDNA analyses by 2 laboratories. Since the 5 two polypeptide sequences show 92% homology, 16 of the synthetic peptides were shared between the panels (numbering from the amino terminus, peptides 1-5, 8, 9, 14, 21, 28, 29, 37-39, 41 and 42). In order to ensure purity, each panel was synthesized by fluorenylmethoxycarbonyl chemistry on resin 10 using a base-labile linker, rather than by convention pin technology, and randomly selected peptides were screened by HPLC and amino acid analysis. The peptides were used to stimulate cultures at 20 μ g/ml, although it should be noted that the responses of the cultures had previously been shown 15 to be similar in magnitude and kinetics at peptide concentration between 5-20 μ g/ml.

The control antigens *Mycobacterium tuberculosis* purified protein derivative (PPD) (Statens Seruminstut, Denmark) and 20 keyhole limpet hemocyanin (KLH) (Calbiochem-Behring, La Holla, Ca., USA) were dialysed extensively against phosphate buffered saline pH 7.4 (PBS) and filter sterilized before addition to cultures at 50 μ g/ml, PPD, but not KLH, readily provokes recall T-cell responses *in vitro*, since most UK citizens have been 25 immunized with BCG. Concanavalin A (Con A) was obtained from Sigma, Poole, Dorset, UK, and used to stimulate cultures at 10 μ g/ml.

Antibodies

30

FITC- or phycoerythrin-conjugated mAbs against human CD3, CD19, CD45 or CD14 were obtained from Dako UK Ltd. Blocking mAbs specific for 11LA-DP, -DQ, or -DR supplied by Becton

Dickinson (Oxford, UK) were dialysed thoroughly against PBS before addition to cultures at the previously determined optimum concentration of $2.5\mu\text{g}/\text{ml}$.

5 Isolation of Splenic or Peripheral Blood Mononuclear Cells and T-cells

Splenic mononuclear cells (SMC) were obtained from homogenised spleen tissue by centrifugation on Ficoll-Hypaque (Sigma) and 10 stored frozen under liquid nitrogen until needed. Peripheral blood mononuclear cells (PBMC) were separated from fresh blood samples using Ficoll-Hypaque. The viability of SMC and PBMC was greater than 90% in all experiments, as judged by trypan blue exclusion. T-cells were isolated from SMC or PBMC by 15 passage through glass beam affinity columns coated with human IgG/sheep anti-human IgG immune complexes. Flow cytometry (Becton Dickinson FACScan) demonstrated that typical preparations contained more than 95% T-cells.

20 Cell Proliferation Assays

SMC or PBMC were cultured in $100\mu\text{l}$ volumes in microtitre plates at a concentration of 1.25×10^6 cells/ml in an Alpha Modification of Eagle's Medium (ICN Flow, Bucks UK) 25 supplemented with 5% autologous serum, 4mM L-Glutamine (Gibco, Paisley, UK), 100U/ml sodium benzylpenicillin G (Sigma), 100 $\mu\text{g}/\text{ml}$ streptomycin sulphate (Sigma), $5 \times 10^{-5}\text{M}$ 2-mercaptoethanol (Sigma) and 20mM HEPES pH7,2 (Sigma). All plates were incubated at 37°C in a humidified atmosphere of 30 5% CO_2 /95% air. The cell proliferation in cultures was estimated from the incorporation of ^{3}H -thymidine in triplicate wells 5 days after stimulation with antigen as described previously. Purified T-cells were cultured under

similar conditions at 1.25×10^6 cells/ml, together with unfractionated MC, which had been irradiated with 2000 rads to prevent their proliferation, and which were added to the wells at a final concentration of 0.6×10^6 cells/ml to act as 5 antigen presenting cells (APC). In some experiments, these cultures were performed in 2ml wells and the incorporation of ^3H -Thymidine was measured in triplicate 100 μl samples withdrawn from the plates over the period 4-9 days after stimulation. Proliferation results are presented either as 10 the mean CPM +/- SD of the triplicate samples, or as a stimulation index (SI), expressing the ratio of mean CPM in stimulated versus unstimulated control cultures. An SI>3 with CPM>500 is interpreted as representing a positive response.

15 As shown in Figure 2 various peptide fragments have been selected in accordance with their particular peptide sequences. These are given in Tables 1, 2 and 3 which follow and the results achieved by means of the foregoing example are shown in Figure 2.

20

Accordingly we have shown that helper T-cells from all donors deliberately immunized against RhD can be stimulated *in vitro* by RhD peptides. Further there is a variation between alloimmune donors in the T-cell response profile to the RhD 25 peptides.

Despite variations, RhD peptides Nos. 2, 6, 12, 12A, 15A, 18A, 28 and 39 are most commonly targeted. However significantly related profiles are found in donors as sharing HLA-DR 30 alleles.

It follows that the characterization of the putative helper T-cell epitopes we have identified is a key step in the

development of safe immunogens for anti-immunoglobulin donors and opens the way to the evaluation of peptide immunotherapy as a novel approach to the prevention of haemolytic disease *inter alia* in neonates.

Table 1

PEPTIDE NUMBER	PEPTIDE SEQUENCE	RESIDUES
RhCE (R2 cLE)		
1	SSKYPRSVRRC1PLW	2 - 16
2	C1PLWALTLEAALIL	12 - 26
3	AALILLFYFFTHYDA	22 - 36
4	THYDASLEDQKGLVA	32 - 46
5	KGLVASYQVGQDLTV	42 - 56
6	QDLTVMAALGLGFLT	52 - 66
7	LGFLLTSNFRRIHSWSS	62 - 76
8	HWSVVAFNLFMLAL	72 - 86
9	FMLALGVQWAILLDG	82 - 96
10	ILLDGFLSQFPPGKV	92 - 106
11	PPGKVVITLFSIRIA	102-116
12	SIRLATMSAMSVLIS	112-126
13	SVLIISAGAVLGKVNL	122-136
14	GKVNI.AQLVVMVLVE	132-146
15	MVLVEVTALGTLRMV	142-156
16	TJLRMVISNIFNTDYH	152-166
17	NTDYHMNLRHFYVFA	162-176
18	FYVFAAYFGLTVAWC	172-186
19	TVAWCLPKPLPKGTE	182-196
20	PKGTEDNDQRATIPS	192-206
21	ATIPSLSAMLGALFL	202-216
22	GALFLWMFWPSVNSP	212-226
23	SVNSPLLRSPIQRKKN	222-236
24	IQRKNAMFNTYYALA	232-246
25	YYALAVSVVTAISGS	242-256
26	AISGESLAHPQRKIS	252-266
27	QRK1SMTYVIHSAVLA	262-276
28	SAVLAGGVAVGTSCH	272-286
29	GTSCHLIPSPWLAMV	282-296
30	WLAMVLGLVAGLISI	292-306
31	GIJSIGGAKCLPVCC	302-316
32	LPVCCCNRVLGIIHHIS	312-326
33	IHHISVMHSIFSSLGG	322-336
34	FSLLGLLGEITYIVL	332-346
35	TYIVIILVLIHTVWNGN	342-356
36	VWNNGNGMIGFQVLLS	352-366
37	QVLLSIGELSLAIVJ	362-376
38	LAIVIALTSGLLTGL	372-386
39	LLTGLLLNLKIWKAP	382-396
40	IWKAPHVAKYFDDQV	392-406
41	FDDQVFWKFPHLAVG	402-416
42	DDQVFWKFPHLAVGF	403-417

Table 2

RhCE (R1 Ce)		
1 (C)	SSKYPRSVRRCLPLC	2 -16
2 (C)	CLPLCALTLEAALIL	12 -26
22 (e)	GALFLWMFWPSVNSA	212-226
23 (e)	SVNSALLRSPIQRKN	222-236
RhD		
6 (also C)	QDLTVMAAIGLGFLLT	52 -66
7 (also C)	LGFITSSFRRHWSWSS	62 -76
10 (also C)	ILLDGFLSQFPSGKV	92 -106
11 (also C)	PSGKVVITLFSIRLA	102-116
12	SIRLATMSALSVLIS	112-126
13	SVLISVDAVLGKVNL	122-136
15	MVLVEVTALGNLRMV	142-156
16	NLRMVISNI FNTDYH	152-166
17	NTDYHMNMHHIYVFA	162-176
18	IYVFAAYFGLSVAWC	172-186
19	SVAWCLPKPLPEGTE	182-196
20	PEGTEDKDQTATIPS	192-206
22	GALFLWIFWPSFNSA	212-226
23	SFNSALLRSPIERKN	222-236
24	IERKNAVFTNTYYAVA	232-246
25	YYAVAVSVVTAJSGS	242-256
26	AISGSSLAH PQGKIS	252-266
27	QGKISKTYVHSAVLA	262-276
30	WLAMVLGLVAGLI SV	292-306
31	GLISVGGA KYLPGCC	302-316
32	LPGCCNRVLGIPHSS	312-326
33	I PHSSIMGYNFSLLG	322-336
34	FSLILGLLGEIIYJVL	332-346
35	IYIVLILVLDTVGAGN	342-356
36	VGAGNGMIGFQVLLS	352-366
40	IWKAPHEAKYFDIQV	392-406
22 (alternative)	GALFLWMFWPSFNSA	212-226

Table 3

RhCE (R1 Ce)

1A (C)	RSVRRCLPLCALTLE	7 -21
22A(e)	WMFWPSVNSALLRSP	217-231

RhD

6A (also C)	MAAIGLGFLTSSFRR	57 -71
7A (also C)	SSFRRHSWSSVAFNL	67 -81
10A(also C)	FLSQFPSGKVITLF	97 -111
11A(also C)	VITLFSIRIATMSAL	107-121
12A	TMSALSVLISVDAVL	117-131
13A	VDAVLGKVNLAQLVV	127-141
15A	VTALGNLRMVISNIF	147-161
16A	ISNIFNTDYHMNMHH	157-171
17A	MNMMHIYVFAYFGL	167-181
18A	AYFGLSVAWCLPKPL	177-191
19A	LPKPLPEGTEDKDQT	187-201
20A	DKDQTATIPSLSAM	197-211
21A	LSAMI.GALFLWIFWP	207-221
22A	WIFWPSFNSALLRSP	217-231
23A	LLRSPIERKNNAVFT	227-241
24A	AVENTYYAVAVSVVT	237-251
26A	SIAHPQGKISKTYVH	257-271
27A	KTYVHSAVLAGGVAV	267-281
30A	LGLVAGLISVGGAKY	297-311
31A	GGAKYLPGCCNRVLG	307-321
32A	NRVLGIPHSSIMGYN	317-331
33A	IMGYNFSLLGLLGEI	327-341
34A	LLGEIIYIVLLVLDT	337-351
35A	LVLDTVAGNGMIGF	347-361
39A	LLNLKJWKAPHEAKY	387-401
40A	HEAKYFDDQVFWKFP	397-411
21A (alternative)	LSAMLGAI.FLWMFWP	207-221
22A (alternative)	WMFWPSFNSALLRSP	217-231

CLAIMS:-

1. A composition adapted for the prevention of alloimmunization of a subject, said composition comprising an immunologically effective epitope of an RhD or RhCE protein or an immunologically active analogue thereof.
2. A composition according to claim 1 wherein the epitope is selected from at least one of RhD or RhCE protein epitopes selected from numbers 2, 6, 12, 12A, 15A, 18A, 28 and 39 hereinbefore set forth.
3. A composition according to claim 2 including the epitope No. 12A.
- 15
4. A composition adapted for the induction of alloimmunization of a volunteer, said composition comprising an immunologically effective epitope of an RhD or RhCE protein or an immunologically active analogue thereof disposed in a pharmacologically acceptable injectable vehicle.
5. A composition according to claim 4 wherein the epitope is selected from at least one of RhD or RHCE protein epitopes selected from numbers 2, 6, 12, 12a, 15a, 18a, 28 and 39 hereinbefore set forth.
6. A composition according to claim 4 including the epitope 12a.
- 30 7. A tolerizing peptide fragment disposed in a pharmacologically effective vehicle, said vehicle being adapted for non-injection administration to the subject.

8. A vehicle according to claim 7 adapted for transdermal or transmucosal administration or wherein said vehicle is a formulation with an enteric coating for oral administration.

5 9. A vehicle according to either of claims 7 or 8 wherein the peptide fragment forms an epitope of claims 1 to 3.

10. A method of tolerizing a subject which comprises administering through said subject a tolerized peptide
10 fragment according to any one of claims 7 to 9.

11. An epitope selected from an RhD or RhCE protein and selected from fragment Nos. 2, 6, 12, 12A, 15A, 18A, 28 and 38 hereinbefore set forth.

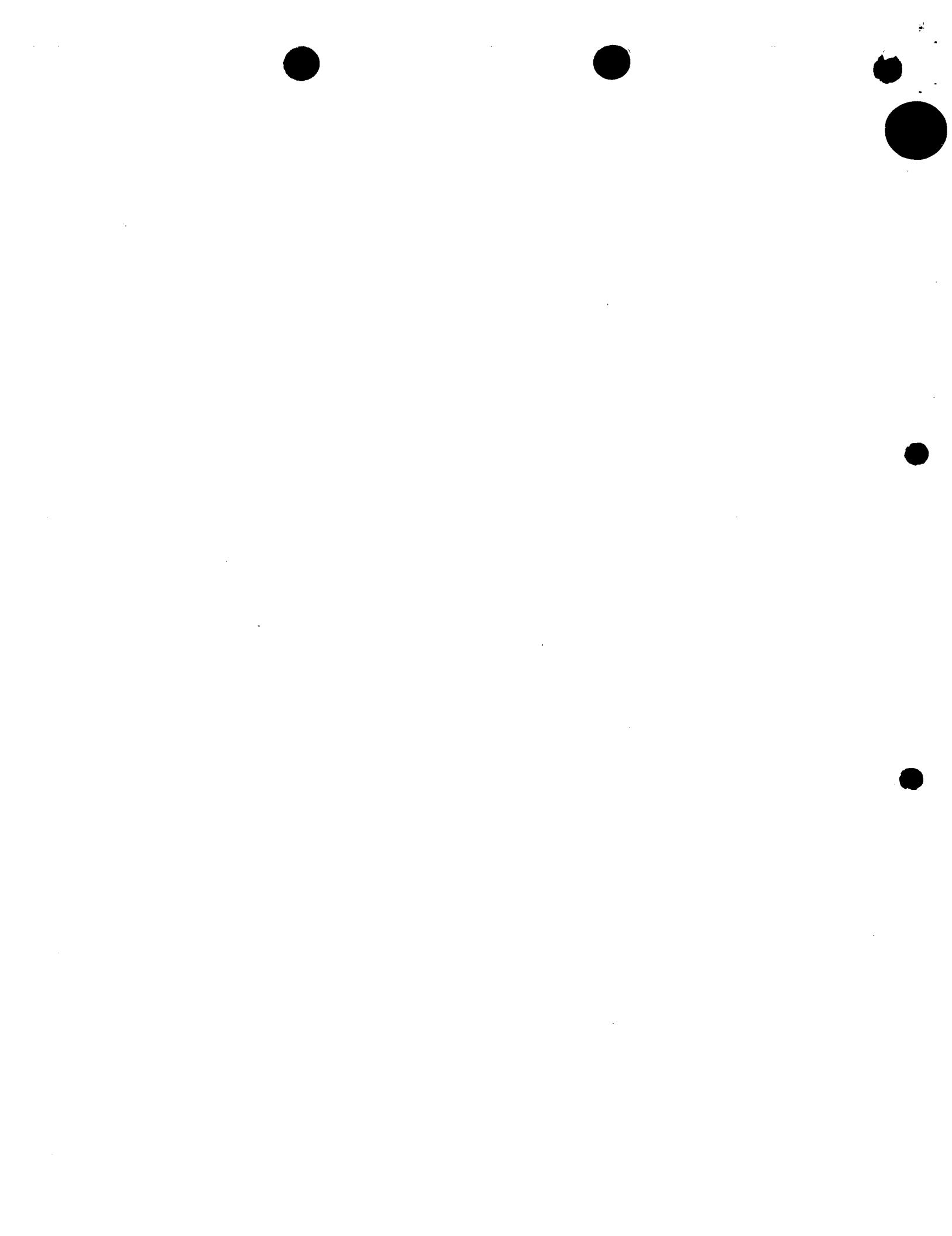
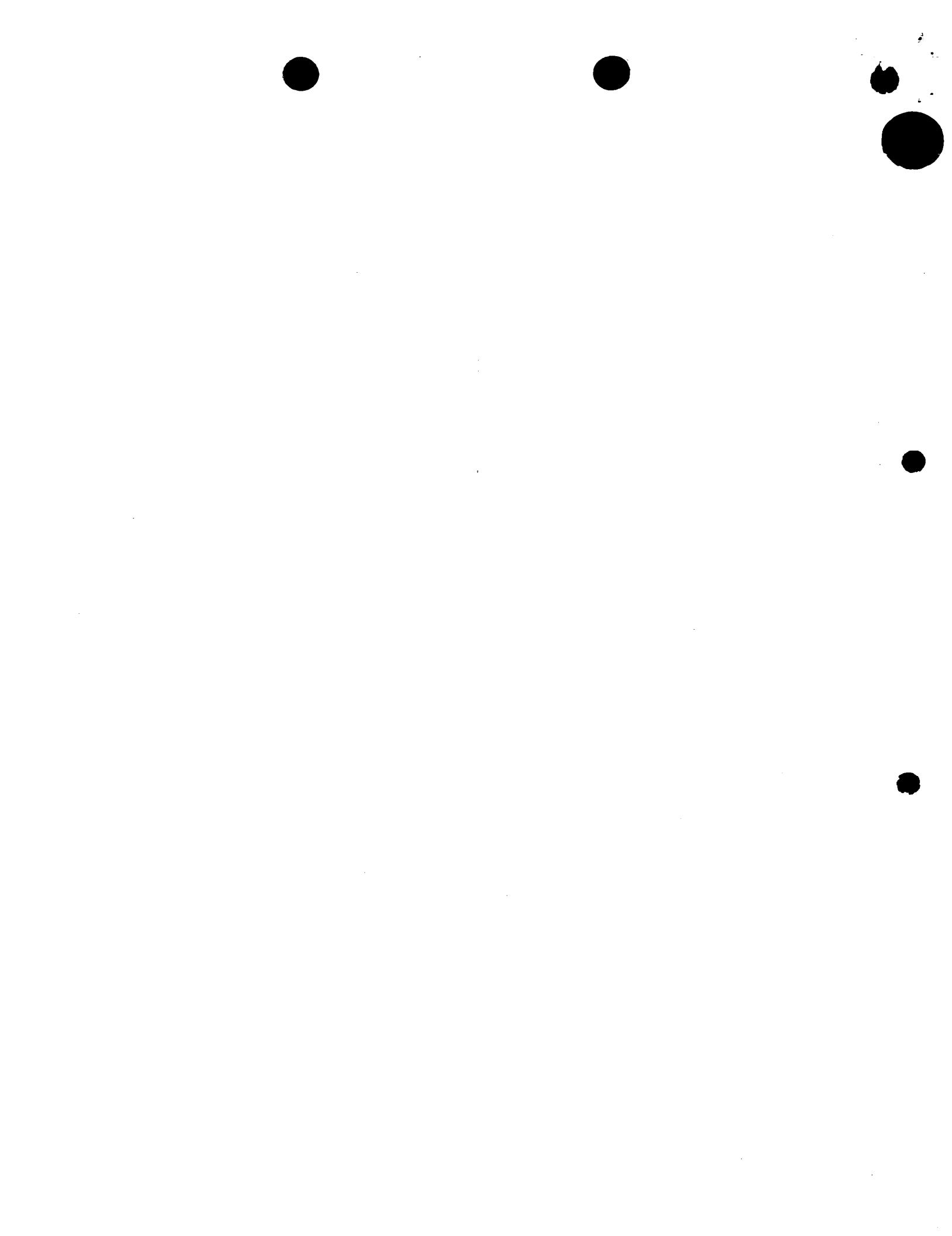
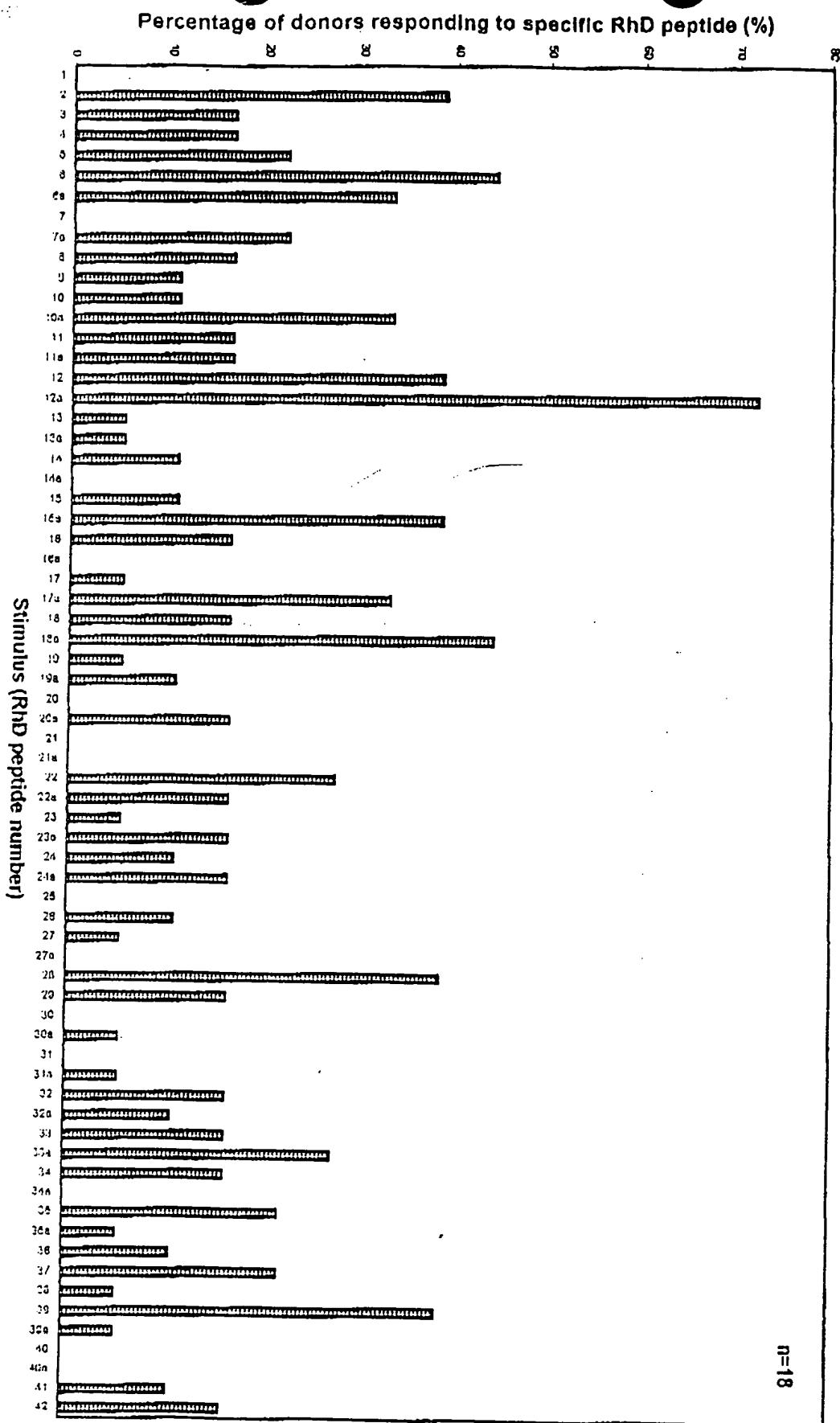


Figure 1

1 / 2

RHC: MSSKYPRSR RCLPLCALTL EAALLLFYF FTHYDASLED QKGLVASYQV 50
RHC:
RHD: W
RHC: GQDLTVMAAI GLGFLTSSFR RHSSSSVAFN LFMALGVQW AILLDGFLSQ 100
RHD: L N
RHC: I S
RHC: FPSGKVWVITL FSIRLATMSA MSVLISAGAV LGKVNLQLV VMLVEVTAL 150
RHD: P
RHD: S
RHC: GTLRMVISNI FNTDYHMNLR HFYVFAAYFG LTVAWCLPKP LPKGTEDNDQ 200
RHD: N MM I S E
RHE: RATIPSLSAM LGALFLWMFW PSVNSPLRS PIQRKNAMFN TYVALAVSVV 250
RHe:
RHD: T I F A E V V
RHC: TAISGSSLAH PQRKISMTRYV HSAVLAGGVA VGTTSCHLIPS PWLAMVGLV 300
RHD: G K
RHC: AGLISIGGAK CLPVCCNRVL CIHHISVMHS IFSLIGLLGE ITYIVLLVLH 350
RHD: V Y G P S I G Y N I D
RHC: TWNGNGMIC FQVLLSIGEL SLAIVIALTS GLTGLLLNL KIWKAAPHVAK 400
RHD: GA
RHC: YFDDQVFWKF PHLAVGF
RHD:





Distribution of stimulatory RhD peptides in anti-RhD immunoglobulin donors.

Figure 2.

PCT/GB399/04027

21/12/98 c?

Ablett + Strabbing